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# Histological Morphology of Gill, Skin and Liver of Nile Tilapia *Oreochromis niloticus* Fingerling Exposed to *Jatropha Curcas* (Linnaeus 1779) Seed Powder

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Adekunle Ajasin University, Akungba Akiko, Ondo State, Nigeria**Abstract:**

The effect of *Jatropha curcas* seed powder on the histopathology of selected organs of *Oreochromis niloticus* fingerlings was investigated. *O. niloticus* fingerlings of 6.5cm-10cm in length and 14.7g-18g in weight were exposed to different concentrations of *J. curcas* seed powder at 133.3mg/l, 200mg/l, 266.7mg/l, 333.3mg/l and 400mg/l. The acute toxicity of the toxicant to *O. niloticus* fingerlings was carried out using bioassay method under laboratory condition and the 96h  $LC_{50}$  was 439.23mg/l with maximum admissible concentration of the toxicant to be 4.3923mg/l – 43.923mg/l which is derived by multiplying the 96h  $LC_{50}$  with an application factor of 0.01 – 0.1. After 96hr of exposure, histopathological changes of gill, liver and skin were observed. In the gill there was degeneration of the gill arch, lamellae are severely stunted, shortened and high degeneration of gill arch, filament & lamellae and severe submucosal congestion, secondary lamellae are absent at the highest concentration. In the skin there was necrosis of the skin and hypertrophy, necrosis and erosion of dermal cell. In the liver disorientation of the liver parenchyma structures, hyperplasia and disorientation of hepatic cells and marked sinusoidal congestion was observed. The pattern of response of *O. niloticus* fingerlings at the various concentration of *J. curcas* seed powder varied widely at the exposure duration. The observable effect of the toxicant on the examined organs could serve as a baseline information needed to develop models of *J. curcas* seed effects on ecological systems.

**Keywords:** Histopathology, *oreochromis niloticus*, *jatropha curcas* seed, gill, skin, and liver

## 1. Introduction

Fish farming or aquaculture has been described as the world's fastest growing food production system and has a lot been identified as a practical and promising approach to meeting the fish demand outside actual fishing (Abalaka and Auta, 2010). However, this sector is facing some challenges, thereby making fishing the only viable alternative where both conventional and unconventional methods are employed for the sole aim of obtaining fishes from the vast water bodies for human consumption. This includes the exploitation of synthetic compounds which bio-accumulate and persist in both target and non-target animals with the result of great effect on man and aquatic environment (Abalaka and Auta, 2010).

The main challenge facing aquatic environment is pollution and this has caused enormous havoc on the organisms in the environment especially their immune system, example of such an aquatic organism is fish which is continuously affected by periodic or unexpected changes of their environment. Adverse environmental situations may acutely or chronically stress the health of fish, altering some of their biochemical parameters and suppressing their innate and adaptive immune responses (Giron-peres *et al.*, 2007).

The health of fish can better be assessed by the histological changes of the organs and the effects of pollution on the biochemical parameters. The histological changes that occur in the organs of fish have been integrated with the impact of various stressors (microbial pathogens, toxic compounds, nutritional and adverse environmental conditions).

*Jatropha curcas* plant (Euphorbiaceae) is a multipurpose plant used for demarcations, construction of gardens, fence and traditional uses. It contains a variety of biologically active phytochemicals such as proteins, peptides and diterpenes exhibiting a spectrum of biological activities (Devappa *et al.*, 2010, 2011). Despite all these attributes, the seeds contain toxic phytochemicals known as phorbol esters (PEs) (Haas *et al.*, 2002; Rakshit *et al.*, 2011).

*Jatropha curcas* L. is an oil-bearing shrub, widely distributed in Nigeria and many Latin American, Asian, and African countries (Gubitz *et al.*, 1999). It is majorly cultivated in developing countries.

Recently, the non-edible *Jatropha curcas* has been hailed as one of the world's most sustainable biofuel crops. The *Jatropha* seed oil has gained tremendous interest as a feedstock for biodiesel production (Makkar and Becker, 2009; Devappa *et al.*, 2010a). Among the different species of *Jatropha*, *Jatropha curcas* has a wide range of uses and promises various significant benefits to human and industry. Extracts from this species have been shown to have anti-tumor activity, the leaves can be used as a remedy for malaria and high fever (Gubitz *et al.*, 1999; Henning, 1997) the seeds can be used in treatment of constipation and the sap was found effective in accelerating wound healing procedure (Gubitz *et al.*, 1999).

Species of the genus *Jatropha* are known to be very toxic. Several cases of *J. curcas* nut poisoning in humans after accidental consumption of seeds have been recorded. Symptoms such as giddiness, emesis and diarrhea have been reported (Makkar & Becker, 1998). *Jatropha* is a drought resistant crop that has a life expectancy of up to 50 years (Koh *et al.*, 2011).

Nile tilapia is a tropical species that prefers to live in shallow water. The lower and upper lethal temperatures for Nile tilapia are 11-12 °C and 42 °C, respectively, while the preferred temperature ranges from 31 to 36 °C. *Oreochromis niloticus* is a relatively large cichlid fish, which is native to Africa from Egypt south to East and Central Africa and as far west as Gambia. It is also native to Israel, and numerous introduced populations exist outside its natural range (e.g. Brazil). According to Fagbenro (2002), it is presently farmed in the tropics, and sub-tropics of all continents, and occasionally where warm water is available, such as thermal effluent or geo-thermal spring.

During the raining season the rivers, sea, ponds and other aquatic environments are always filled with floods which in most cases comes with increase in volume and turbidity of water. The seeds of *J. curcas* comes along with the runoff from the gardens and farms into the rivers and ponds. In this case the seed can be swallowed by adult fish or crushed by some amphibians and crustaceans which assist in the release of the toxic substances such as the phorbol ester, the saponins and the curcins that can be responsible for the toxification of the aquatic environment.

## 2. Materials and Methods

The experiment was conducted under standard static bioassay procedure (Reish and Oshida, 1987; American Public Health Association, 1987). Which involved carefully controlled environmental conditions as to define the response of the test fishes to *J. curcas* seed powder. The experiment was conducted at the Fish Farm (Obakekere) of the Federal University of Technology, Akure.

### 2.1. Collection of Experimental Fishes

Apparently healthy *O. niloticus* fingerlings (6.5cm-10cm, weighing 14.7g-18g) were collected from Ondo State Agricultural Development Project (ADP) farm, Akure Ondo State, Nigeria, and acclimated for one week in the farm inside rectangular concrete tanks, (75cm x 45cm x 45cm) container of 121.5 litres capacity, filled with 50litres unchlorinated well water. The fish were fed with pelleted fish diet containing 40% crude protein, during the acclimation period. Feeding was discontinued after 48 hours before the commencement of the experiment to minimize the production of waste in the test container.

### 2.2. Preparation of *Jatropha curcas* Seed Powder

Large quantities of freshly mature seeds of *J. curcas* were collected from a private garden at No.12 Clerk Quarters, Owo, Nigeria. The seed powder was prepared according to the method described by Price (2000). The seeds were sun-dried at ambient temperature (25-28°C over three days), seed coats and wings were manually removed. The white kernel was ground to fine powder, using the coffee mill attachment of a Moulinex domestic blender. The powder was kept in the freezer in an air tight cellophane bag for later use. The behaviour pattern and mortality of the test fishes in each tank was monitored and recorded every 15 minutes for the first hour, once every hour for the next three hours and every four hours, for the rest of the 24 hours, and once every 24 h until 96 h. Dead fish were removed immediately with a scoop net.

### 2.3. Toxicity Test

#### 2.3.1. Range Finding Test

Preliminary 24-hours range finding test was conducted for the experimental fishes following static bioassay procedures described by Parrish (1985) to determine the toxic range of *J. curcas* seeds to the experimental fishes. There were six treatments comprising three replicates, hence 36 tanks were used. A batch of ten of each of the experimental fishes were batch weighed and stocked into each tank (75cm x 45cm x 45cm) filled with 30L of unchlorinated well water. The seed powder was introduced into each tank at 400mg/l, 466.67mg/l, 533mg/l, 600mg/l and 666.67mg/l with a control of 0 mg/l. The behaviour and mortality of the test fish in each tank were monitored and recorded every 15 minutes for the first hour, once every hour for the next three hours and every four hours for the rest of the 24hours period.

#### 2.3.2. Definitive Test

Based on the results from the range finding (Lethal toxicity) test described above, 96 hours definitive (sub-lethal toxicity) tests following static bioassay procedures described by Parrish (1985) was carried out. There were six treatments comprising three replicates, hence 18 tanks were used. A batch of ten of each of the experimental fishes were batch weighed and stocked into each tank (75cm x 45cm x 45cm) each filled with 30L of unchlorinated well water. The seed powder was introduced into each tank at 133.3mg/l, 200mg/l, 266.7mg/l, 333.3mg/l and 400mg/l. Test fish were not fed throughout the 96hours that the experiment lasted. Mortality of the test fish in each tank were monitored and recorded every 15 minutes for the first hour, once every hour for the next three hours, every four hours for the next 24 hours and every 24hours for the remaining 96 hours. The inability of fish to respond to external stimuli was used as an index of death. Dead fish were removed immediately with a scoop net to avoid contamination due to rotting. Apart from monitoring and recording mortality, the fish behaviour such as erratic swimming, air gulping and loss of reflex were also monitored.

## 2.4. Histological Examination of Test Organs

At the end of the experiment, one fish per treatment, that is, three fish per concentration were sampled after 96 hours of exposure to *J. curcas* seed powder for histological analysis, the fish was anesthetized in aerated buffered tricaine methanesulfonate (MSS 222) to depress the CNS so as to reduce the impact of pain. The fish was sacrificed and dissected to remove the gill, liver and skin. The organs were fixed in 100% formalin for three days after which the tissue was dehydrated in periodic acid Schiff's reagent (PAS) following the method of Hughes and Perry (1976). In graded levels of 50%, 70%, 90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding.

The organs were then embedded in molten wax. Tissue were sectioned into thin sections (5-7  $\mu$ m), by means of a rotatory microtome and were dehydrated and stained with harris haematoxylin-eosin (H&E) stain, Bancroft and Cook (1994), using a microtome and each section was cleared by placing in warm water (38°C), where it was picked with clean slide and oven-dried at 58°C for 30 minutes to melt the wax. Slides containing sectioned materials/tissue was cleared using xylene and graded levels of 50%, 70%, 90%, 95% and 100% alcohol for two minutes each.

The section was stained in haematoxyline eosin for ten minutes. The stained slide were observed under a light microscope at varying X100 magnification, sections were examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus C35 AD4), a digital camera (Olympus C40 AB-4) and an automatic light exposure unit (Olympus PM CS5P).

## 3. Result and Discussion

The 96h LC<sub>50</sub> obtained for the fingerlings of *O. niloticus* was 439.23mg/l. The maximum admissible concentration of toxicant to the fish was established to be 4.3923mg/l – 43.923mg/l which is derived by multiplying the 96h LC<sub>50</sub> with an application factor of 0.01 – 0.1.

The histological changes observed in *O. niloticus* fingerlings exposed to 133.3, 200, 266.7, 333.3, 400mg/l and 0mg/l as control is presented in Table 1 and Figures 1-3. Histological changes of gill, skin and liver was observed for all the treatments. Intensity of cell damage increased with increase in concentration.

Concentration(Mg/L)	Gill	Skin	Liver
CONTROL	No visible lesions	Normal skin, No visible lesion.	No lesions observed.
133.3	No visible lesions	Normal skin, No visible lesion.	Disorientation of the liver parenchyma structures.
200	Degeneration of the gill arch	Normal skin, No visible lesion.	Disorientation of the liver parenchyma structures.
266.7	Lamellae are severely stunted	Normal skin, No visible lesion.	Hyperplasia and disorientation of hepatic cells.
333.3	Shortened and High degeneration of gill arch, filament and Lamellae.	Necrosis of the skin	Marked sinusoidal congestion
400	Severe submucosal congestion, secondary lamellae are absent.	Hypertrophy, Necrosis and erosion of dermal cell of the skin.	Severe degeneration of hepatocyte

Table 1: Summary of Histological Changes Observed in *O. Niloticus* Finger Lings Exposed to *Jatropha Curcas* Seed Powder

Figures 1 to 6 Histological changes observed in the gill of *O. niloticus* fingerlings exposed to *J. curcas* seed powder for 96h.

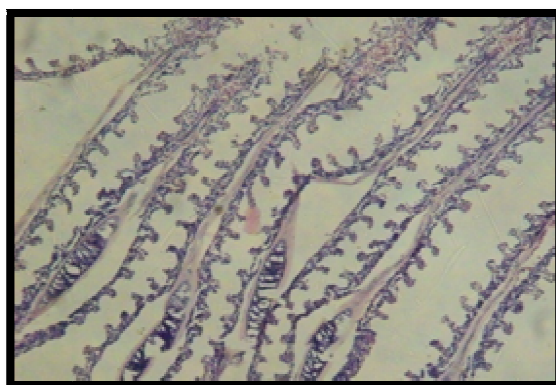
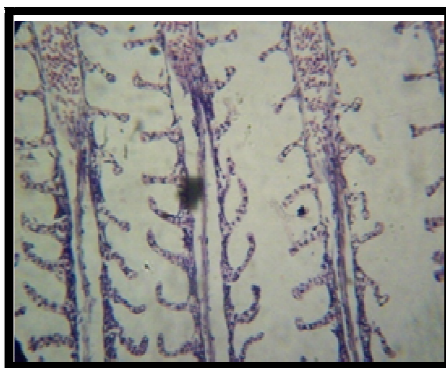
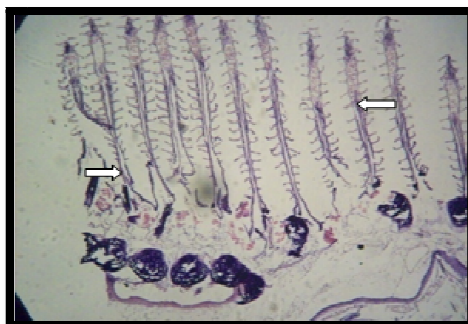


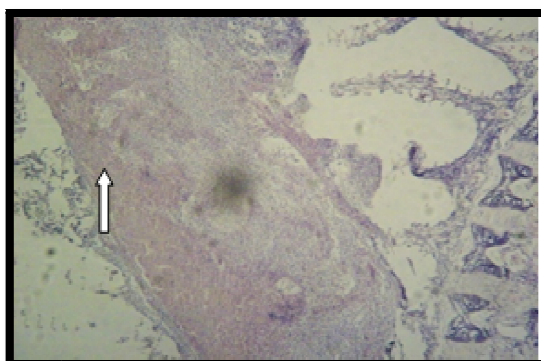
Figure 1: Gill of Fingerlings *O. Niloticus* in the Control Tank with No Lesion (X100)



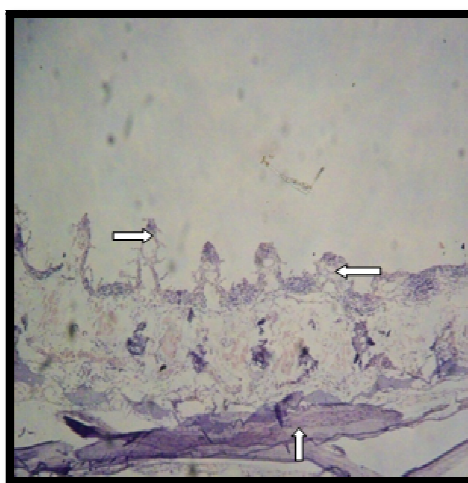
*Figure 2: Gill of Fingerlings O. Niloticus Exposed to 133.3mg/L Showing No Lesion (X100)*



*Figure 3: Gill of Fingerlings O. Niloticus Exposed to 200mg/L Of J. Curcas Showing Degeneration on the Gill Arch. (X100)*



*Figure 4: Gill Of Fingerlings O. Niloticus Exposed To 266.7mg/L Of J. Curcas Showing Severely stunted Lamellae(X100)*



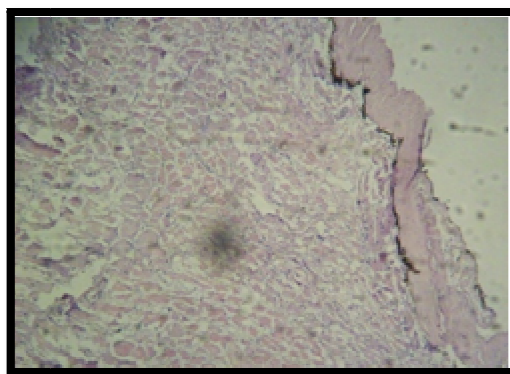
*Figure5: Gill of Fingerlings O. Niloticus Exposed To 333.3mg/L off. Curcas Showing Shortened and High Degeneration of Gill Arc, Filament and Lamellae. (X100)*



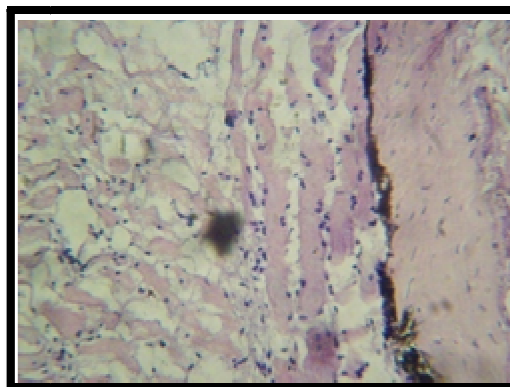


*Figure 6: Gill of Fingerlings O. Niloticus Exposed To 400mg/L off. Curcas Showing Severe submucosal Congestion and Absence of Secondary Lamellae (X100)*

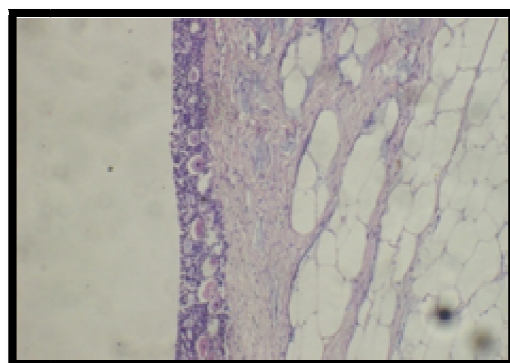
Figures 7 to 12 Histological Changes Observed in The Skin Of O. Niloticus Fingerlings Exposed to Different Concentration of J. Curcas Seed Powder.



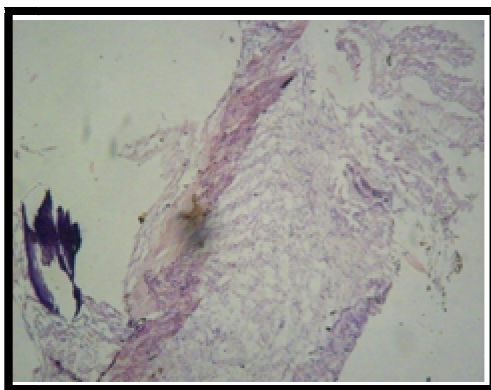
*Figure7: Skin of Fingerlings O. Niloticus in the Control Tank with No Visible Lesion. (X100)*



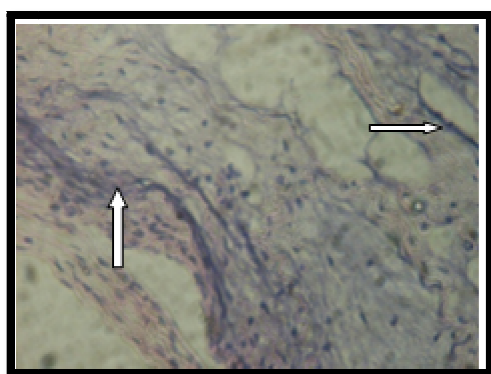
*Figure8: Skin of Fingerlings O. Niloticus in the Exposed To 133.3mg/L off. Curcas. No Visible Lesion in the Skin. (X400)*



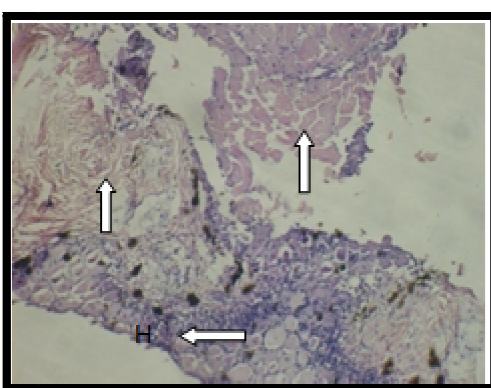
*Figure9: Skin of Fingerlings O. Niloticus in the Exposed To 200mg/L off. Curcas. No Visible Lesion in the Skin. (X100)*



*Figure 10: Skin of Fingerlings O. Niloticus in the Exposed To 266.7mg/L of J. Curcas. No Visible Lesion in the Skin (X100).*

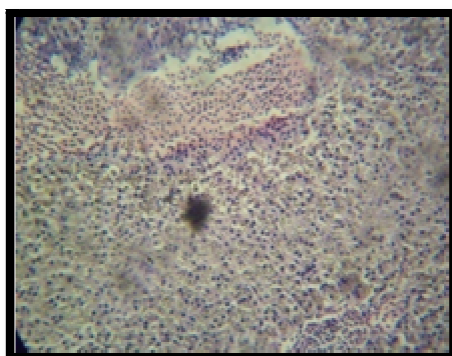


*Figure 11: Skin Of Fingerlings O. Niloticus In The Exposed To 333.3mg/L Of J. Curcas Showing Necrosis Of The Skin*

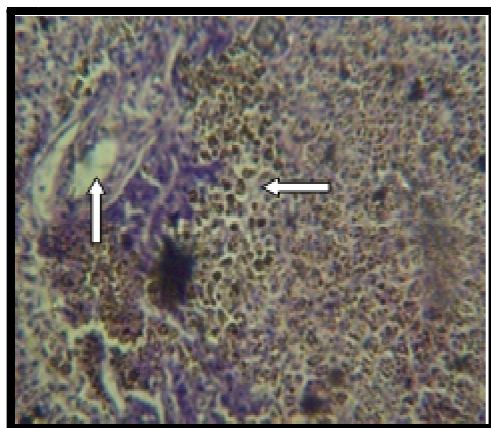


*Figure 12: Skin Of Fingerlings O. Niloticus in The Exposed To 400mg/L Of J. Curcas showing Hypertrophy, Necrosis And Erosion Of Dermal Cell Of The Skin.*

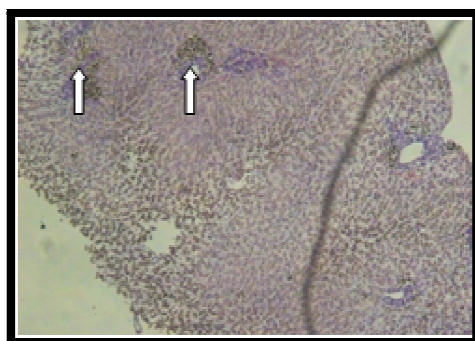
Figure 13 to 18 Histological Changes Observed In The Liver Of Fingerlings O. Niloticus Exposed To J. Curcas Seed Powder At 96h.



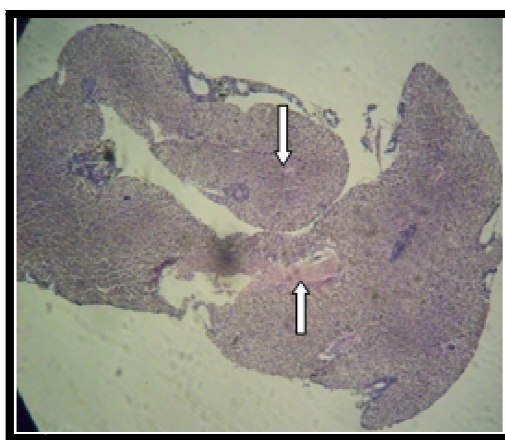
*Figure 13: Liver Of Fingerlings O. Niloticus in The Control Tank with No Visible Lesion. (X100)*



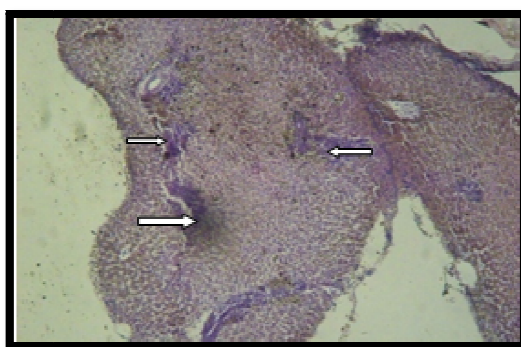
*Figure14: Liver of Fingerlings O. Niloticus exposed to 133.3mg/L Of J. Curcas Showing Necrosis and Disorientation of the Liver Parenchyma Structures (X400)*



*Figure 15 : Liver Of Fingerlings O. Niloticus exposed To 200mg/L Of J. Curcas Showing Disorientation of the Liver Parenchyma Structures,(X100)*



*Figure16: Liver of Fingerlings O. Niloticus exposed to 266.7mg/L Of J. Curcas Showing Hyperplasia and Disorientation of Hepatic Cells. (X100)*



*Figure17: Liver Of Fingerlings O. Niloticus exposed To 333.3mg/L Of J. Curcas Showing Marked Sinusoidal Congestion. (X100)*

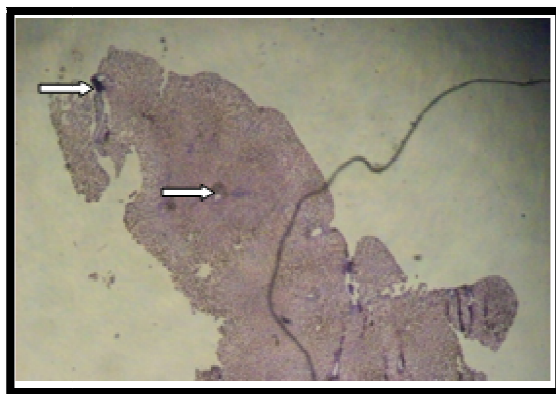


Figure18: Liver of Fingerlings *O. Niloticus* exposed to 400mg/L Of *J. Curcas* Showing Severe Degeneration of Hepatocyte (X100)

#### 4. Discussion

The values of the toxicity of *J. curcas* seed powder to the fingerlings of *O. niloticus* after 96h of exposure reveal that the 96h LC<sub>50</sub> is 439.23mg/l with maximum toxicant admissible concentrations of 4.3923 - 43.923mg/l which is derived by multiplying the 96h LC<sub>50</sub> with a constant value of 0.01- 0.1 according to Koesoemadinata (1980).

Ayotunde *et al.* (2011) reported lower concentration of the aqueous extract of *Moringa olifera* seed powder 96h LC<sub>50</sub> of 242mg/l for *O. niloticus* fingerlings. Values of toxicity of *J. curcas* to *O. niloticus* is higher than the values obtained by Ayotunde and Ofem (2008) who reported 96h LC<sub>50</sub> of 1.8mg/l, for *O. niloticus* exposed to the seed powder of *Carica papaya*. The value in this study is also higher than that of Ringworm plant *Senna alata*, used in poisoning water bodies for fish capture in Benue State of Nigeria, the 96h LC<sub>50</sub> for juvenile *O. niloticus* was 13.93mg/l, Indicated that the extract causes sub acute effect. Ayoola (2011) obtained LC<sub>50</sub> after 96 h of exposure for aqueous and ethanolic extracts of *Ipomoea aquatica* to be 2.659 and 0.196 g/L, respectively. Similar thing was observed by Agbon (2002); Ayotunde (2010ab); Ada *et al.* (2011), all these shows that *J. curcas* is less toxic to *O. niloticus* than all the toxicant used.

Histological examinations of *O. niloticus* gave significant indication of toxicity of *J. curcas* (Table1, Figures 1-3). The effects include gill alterations such as: Degeneration of the gill arc, Stunted lamellae, Degeneration of filament & lamellae and congestion of submucosal of the gill which denotes gill functional disorders that may affect the fish physiology or cause death of the fish. Damages of the gills indicated impairment in gaseous exchange efficiency of the gills. This is similar to the observation of Ayotunde *et al.* (2011a) on toxicity of aqueous extract of *Moringa Olifera* on the fingerlings of *O. niloticus*. Histological evidence of the gill damaged caused by *J. curcas* toxicity was evident, resulting from malfunctioning of the gill. Francis *et al.* (2002) and Roy *et al.* (1990) reported that saponins an active component of *J. curcas* causes damage on respiratory epithelia of fish and that fish exhibit stress reactions to the presence of saponins in water.

The observations on the skin such as Necrosis, Hypertrophy and erosion of dermal cell could be as a result of the effect of the irritation due to direct contact with the toxicant and the curcanoleic acid contained in *J. curcas* which may promote skin cancer (Silitonga *et al.*, 2011 and Osode *et al.*, 2011). This agreed with the findings of Ayotunde *et al.* (2011a).

The intensity of cell damage increased with increasing concentration and time of exposure. The reactions on the skin could be as a result of paradoxical biological activities of phorbol ester a strong co- carcinogenic component of *J. curcas* seed.

The liver is an important centre of metabolism of various substances and supporting the stability of intra-circumstances of organism, therefore the changes that occur in the liver would interfere with the normal metabolic function of the liver cells. Mortality may be as a result of the disorder of the liver. Changes that occur in the liver include: Disorientation of the liver parenchyma, hyperplasia & disorientation of hepatic cell, sinusoidal congestion and degeneration of hepatocyte of the liver cells and necrosis. This agreed with Ayotunde *et al.* (2011a) which observed disorientation of the liver parenchyma structure, vacuole formation and shrinkages of cell in high concentration of *M. oleifera* treated *O. niloticus* fingerlings.

Degeneration and necrosis of liver hepatocytes may be attributed to the cumulative effect of *J. curcas* and the increase of their concentrations in the hepatic tissue. This result agreed with Forlin *et al.* (1986), who stated that the liver has important detoxical role on endogenous waste products as well as externally derived such as heavy metals. This is similar to what was observed by Ayoola (2011) when *O. niloticus* juveniles were exposed to aqueous and ethanolic extracts of *Ipomoea aquatica* leaf. The death of *O. niloticus* fingerlings could probably be as a result of all the malfunctions in the observed organs of the fish.

The observed effects of *J. curcas* seed powder on some of the organs of *O. niloticus* fingerlings fish had a positive correlation with exposure. Although the effects on the organs could be less when compared with other toxicant but the bioaccumulation effect on these organs of any exposed fish can be potentially hazardous especially the freshwater fish. Therefore, the results of the observations recorded in the present study provide baseline information needed to develop models of *J. curcas* seed effects on ecological systems.



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